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EXAMINER	
LI, QIAN JANICE	
ART UNIT	**PAPER NUMBER**
1632	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/049,825	FRANZ, WOLFGANG M.	
	Examiner	Art Unit	
	Q. Janice Li	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 January 2004* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *34-50* is/are pending in the application.

4a) Of the above claim(s) *47-49* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *34-46,50* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *19 February 2002* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

The response and amendment filed 1/20/04 has been entered. Claims 1-33 have been canceled. Claims 34-50 are newly submitted, and pending in the application.

Restriction and Election

It is noted the elected invention for examination in this application is drawn to an expression cassette and a method of using such for genetically modifying pluripotent stem cells. New claims 34-46 and 50 belong to the original elected invention. In the originally presented claims, the preferred embodiment specifically identified in the independent or dependent claims are drawn to several types (species) of expression cassettes in different combination of following elements: a cardiac muscle-specific promoter that is MLC-2v (claim 14), the extracellular and transmembrane domains of a CD4 receptor, a CMV enhancer, a PGK promoter, therapeutic proteins of an angiogenesis factor and an immunosuppressive factor that is CTLA4-Ig fusion protein.

Newly submitted claims 34-46 and 50 broadly encompass *any* cardiac muscle-specific promoter operably linked to a polynucleotide encoding extracellular and transmembrane domains of *a genus of* receptor expressed by B or T cells which encompasses numerous receptors as recited in new claims 39 to 42, and the polynucleotide further comprises *a genus of* enhancer operative in a mammalian ES cell, primordial cell, or bone marrow stromal cell, *a genus of* promoter constitutively operative in a mammalian ES cell, primordial cell or bone marrow stromal cell, and *a*

genus of immune suppressive protein. As such, these claims encompass a multitude of patentably distinct species of expression cassettes with different utilities, each comprising specific combinations of either or both cardiac specific and ES cell specific promoters, enhancers, receptors, and either or both groups of therapeutic proteins. Depending on the specific elements carried in the recombinant expression cassette, the utility of the vector may be patentably distinct. The claims would have been restricted as different species of an invention if presented originally. Since applicant has received an action on the merits for the originally presented invention. which already encompasses a reasonable number of species, this invention has been constructively elected by original presentation for prosecution on the merits. Accordingly, the subject matter in claims 34-46 and 50 that are not presented originally will be withdrawn from consideration as being directed to a non-elected invention. See 37 CFR 1.142(b) and MPEP § 821.03. These claims would be examined to the extend that read on the originally presented invention as defined in the immediate preceding paragraph.

Newly submitted claims 47-49 are directed to an invention that is independent or distinct from the invention originally claimed for the following reasons: Newly submitted claims 47-49 are directed to any mammalian cells comprising an expression vector, which belong to invention group II as indicated in the office action paper #6. Thus, claims 47-49 are withdrawn from further consideration by the Examiner, pursuant to 37 CFR 1.142(b), as being drawn to non-elected inventions, there being no allowable generic or linking claim. Claims 34-46 and 50 are under current examination.

Unless otherwise indicated, previous objections and rejections that have been rendered moot in view of the amendment will not be reiterated. The arguments in 1/20/04 response would be addressed to the extent that they apply to current rejection.

Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

The prior rejection of claim 32 under 35 U.S.C. 101 is withdrawn because the rejection should not have been made to a method claim.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The prior rejection of claim 32 under 35 U.S.C. 112, first paragraph is withdrawn in view of claim amendment limiting the process to *in vitro*.

Claims 34-46, and 50 are newly rejected under 35U.S.C. 112 first paragraph, because the specification as originally filed does not describe the invention as now claimed. The original disclosure fails to specify a genus of "enhancer operative in a mammalian ES cell, primordial cell or bone marrow stromal cell" or a genus of "a promoter constitutively operative in a mammalian ES cell, primordial cell or bone

marrow stromal cell" as now claimed. These claimed genuses are now considered to be new matter.

MPEP 2163.02 states, "WHENEVER THE ISSUE ARISES, THE FUNDAMENTAL FACTUAL INQUIRY IS WHETHER A CLAIM DEFINES AN INVENTION THAT IS CLEARLY CONVEYED TO THOSE SKILLED IN THE ART AT THE TIME THE APPLICATION WAS FILED...IF A CLAIM IS AMENDED TO INCLUDE SUBJECT MATTER, LIMITATIONS, OR TERMINOLOGY NOT PRESENT IN THE APPLICATION AS FILED, INVOLVING A DEPARTURE FROM, ADDITION TO, OR DELETION FROM THE DISCLOSURE OF THE APPLICATION AS FILED, THE EXAMINER SHOULD CONCLUDE THAT THE CLAIMED SUBJECT MATTER IS NOT DESCRIBED IN THAT APPLICATION". MPEP 2163.06 further notes "WHEN AN AMENDMENT IS FILED IN REPLY TO AN OBJECTION OR REJECTION BASED ON 35 U.S.C. 112, FIRST PARAGRAPH, A STUDY OF THE ENTIRE APPLICATION IS OFTEN NECESSARY TO DETERMINE WHETHER OR NOT "NEW MATTER" IS INVOLVED. *APPLICANT SHOULD THEREFORE SPECIFICALLY POINT OUT THE SUPPORT FOR ANY AMENDMENTS MADE TO THE DISCLOSURE*" (emphasis added). In the instant case, applicants fail to particularly point out where in the specification supports for the new claims could be found. The specification as originally filed discloses a PGK promoter and a CMV enhancer. However, the specification is completely silent with respect to a genus of promoters that constitutively operabtive in a mammalian ES cell, primordial cell or bone marrow stromal cell, the specification fails to teach the association of the PGK promoter with the genus as now claimed, and representative promoters that belong to this genus. Thus, the amendment is a departure from or an addition to the disclosure of the application as filed, accordingly, it introduces new matter into the disclosure. Likewise, the specification is completely silent with respect to a genus of enhancers that are operabtive in a mammalian ES cell, primordial cell or bone marrow stromal cell, the

specification fails to teach the association of the CMV enhancer with the genus as now claimed, or representative enhancers that belong to this genus. Thus, the amendment is a departure from or an addition to the disclosure of the application as filed. Accordingly, the amendment introduces new matter into the disclosure. However, Applicants are invited to point out the pages and lines in the specification where support for the rejected subject matter could be found.

For reasons set forth above, the amendment filed 2/20/04 is objected to under 35 U.S.C. §132 because it introduces new matter into the disclosure. 35 U.S.C. §132 states that no amendment shall introduce new matter into the disclosure of the invention. Applicant is required to cancel the new matter in the reply to this Office Action.

To the extent that the claimed methods are not described in the instant disclosure, claims 34-46 and 50 are also rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention, since a disclosure cannot teach one to make or use something that has not been adequately described.

Moreover, Claims 34 (C), (D), (E-I) are drawn to a promoter constitutively operative in mammalian ES cells (ii) operably linked with a cardiac muscle-specific promoter (i). Hence, the part (ii) ES promoter would not function in ES cells because it

linked to the cardiac muscle-specific promoter nor in differentiated cardiomyocytes because it is ES cell specific. Accordingly, this part of the construct appears never active when used to transfect ES cells, the specification fails to teach how to use such an expression cassette, and thus, fails to provide an enabling disclosure for what is now claimed.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 34-46 and 50 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims are vague and indefinite because claim 34 recites, "an expression cassette comprising polynucleotides" selected from (A)-E), which encompasses a cassette comprising more than one polynucleotides of A-E. Since the polynucleotide constructs described in A-E have overlapping components, yet functions differently or distinctively, it is unclear whether applicants intend to place more than one of them together in the same cassette. Thus, the metes and bounds of the claims are uncertain.

Claims are vague and indefinite because each polynucleotide A-E of claim 34 comprises at least one IRES, yet, its relative location or its relationship with the promoter is uncertain, thus, the metes and bounds of the claims are unclear.

Claims are vague and indefinite because the polynucleotides recited in claim 34 (C), (D), (E-I) are drawn to a promoter constitutively operative in mammalian ES cells (ii)

operably linked with a cardiac muscle-specific promoter (i). Hence, the part (ii) ES promoter would not function in ES cells because it linked to the cardiac muscle-specific promoter nor in differentiated cardiomyocytes because it is ES cell specific. Accordingly, this part of the construct appears never function when used to transfect ES cells, thus, the metes and bounds of the claims are uncertain. For the sake of a compact prosecution and for prior art purpose, they would be interpreted as independent from the cardiac muscle-specific promoter.

The claims are vague and indefinite because of the phrase starting at line 4 of claim 34(c), "operatively linked to a polynucleotide", this is because the subject to which the polynucleotide linked to is unclear, whether it is the enhancer in line 1 or the muscle-specific promoter in line 3, thus, the metes and bounds of the claims are unclear. Deleting the comma after "cardiac muscle-specific promoter" would overcome this rejection.

Claims are vague and indefinite because claim recitation, "a protein that provides a selectable or screenable marker gene". It is unclear how a protein provides a gene, thus the metes and bounds of the claims are unclear.

Claim 45 recites the limitation "the at least one angiogenesis factor". There is insufficient antecedent basis for this limitation in the claim.

Claim Objections

Claim 34 is objected to because "a" should be inserted before "B" in line 6 of claim 34. Appropriate correction is required.

Claim 34 is objected to because the abbreviations "ES" and "IRES" should be spell out the first time they appear in the claims.

Claim 36 is objected to because the abbreviation "PGK" should be spelled out the first time it appears in the claims.

Claim 34 (C) & (D) recites, a promoter operatively linked to a secreted immunosuppressive protein, whereas a promoter could only be operative when it links to a nucleic acid encoding a protein. Further the word "secreted" appear to define the cellular location of a translated protein, i.e. whether it stays insight the cell or out, and it does not meaningfully define the function or the nature of the protein.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

The prior rejection of claims 1-5, 12, and 16 under 35 U.S.C. 102(b) as being anticipated by *Segre et al* (5,494,806) is withdrawn in view of claim amendment limiting the receptor to a lymphocyte receptor.

The prior rejection of claims of 1, 2, 4, 5, 12, and 16 under 35 U.S.C. 102(b) as being anticipated by *Reppert et al* (5,856,124) is withdrawn in view of claim amendment limiting the receptor to a lymphocyte receptor.

The prior rejection of claims of 1, 2, 4, 5, and 16 under 35 U.S.C. 102(b) as being anticipated by *Harlan et al* (5,718,883) is withdrawn in view of claim amendment limiting the receptor to a lymphocyte receptor.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

In view of claim amendment, the rejections under this section have been modified and appear below.

The prior rejection of claims 1-6, 10, 12, 13, 15-17, and 32 under 35 U.S.C. 103(a) as being unpatentable over *Klug et al* (J Clin Invest 1996;98:216-24, IDS), *Gaines et al* (IDS, Biotechniques 1999;26:683-8), in view of *Griscelli et al* (Hum Gene Ther 1998;9:1919-28), and *Wolfgang-M et al* (J Mol Cell Cardiol 1997;29(5):A125) is withdrawn in view of claim amendment.

The prior rejection of claim 9 under 35 U.S.C. 103(a) as being unpatentable over *Klug et al* (J Clin Invest 1996;98:216-24), *Gaines et al* (IDS, Biotechniques

1999;26:683-8), *Wolfgang-M et al* (J Mol Cell Cardiol 1997;29(5):A125), and *Griscelli et al* (Hum Gene Ther 1998;9:1919-28), as applied to Claims 1-6, 10, 12, 13, 15-17, and 32 above, and further in view of *Cheng et al* (Gene Ther 1997;4:1013-22) is withdrawn because the new claims do not require a GFP as the marker protein.

The prior rejection of claims 1-6, 10-13, 14 (a) (b), 15-17, and 32 under 35 U.S.C. 103(a) as being unpatentable over *Klug et al* (J Clin Invest 1996;98:216-24), *Gaines et al* (IDS, Biotechniques 1999;26:683-8), *Griscelli et al* (Hum Gene Ther 1998;9:1919-28), and *Wolfgang-M et al* (J Mol Cell Cardiol 1997;29(5):A125), and in view of *Mack et al* (J Thorac Cardiovasc Surg 1998;115:168-76) now applies to claims 34 (A, B), 35, 38, 39, 40, 42, 46, and 50.

Klug et al teach a method for genetic alteration of cultivated murine embryonic stem cells (pluripotent precursor cells) for selective obtaining cardiomyocytes. The method comprises transforming the ES cells with a vector comprising an expression cassette (abstract), wherein the cassette comprising a cardiomyocyte-specific promoter (α-cardiac myosin heavy chain promoter, MHC) operably linked to two marker gene, neo^r resistance gene and pGK hygromycin sequence, and a polyA regulatory element (paragraph bridging pages 216-7). *Klug et al* teach using such method for selecting cardiomyocytes from differentiating ES cells because the marker gene is driven by the MHC promoter, thus only expressed in differentiated cardiomyocytes. *Klug et al* state, "A SIMPLE GENETIC MANIPULATION CAN BE USED TO SELECT ESSENTIALLY PURE CULTURES OF CARDIOMYOCYTES FROM DIFFERENTIATING ES CELLS. MOREOVER, THE RESULTING

CARDIOMYOCYTES ARE SUITABLE FOR THE FORMATION OF INTRACARDIAC GRAFTS" (abstract). *Klug et al* go on to teach that refinement of the selection procedure should be possible simply by choosing an appropriately cell type-restricted promoter for selecting atrial versus ventricular cardiomyocytes or for selecting conduction system versus working cardiomyocytes (3rd paragraph, page 222). *Klug et al* also teach other cardiomyocyte-specific promoters such as MLC-2v (right column, page 216). *Klug et al* use antibiotic resistance gene for cardiomyocyte selection, and do not teach cell sorting with a poly-cistronic gene cassette including a T/B cell receptor as the selection marker or co-delivering a VEGF gene.

Gaines et al teach a poly-cistronic expression cassette comprising a nucleic acid encoding the extracellular and transmembrane domain of the human CD4 (receptor) and a luciferase marker gene operably linked to a dicistronic IRES, which mediates translational initiation. *Gaines et al* teach that the construct could be used for co-expression of multiple heterologous gene and selecting for cells expressing the heterologous gene (abstract). They transfect myeloid cells with the expression vector, and selecting for cells expressing CD4 surface marker by FACS. Here, CD4 receptor was used as a selection marker as well as a heterologous gene generally for any cell and IRES was used to coordinate multiple gene expression when such is necessary. *Gaines et al* teach that the dicistronic construct is advantages over co-transfected or dual-promoter vectors when it comes for delivering multiple gene because the expression could be coupled (mid-column, page 1), that cells transfected with pIRES-CD4t can be efficiently retrieved and analyzed quantitatively using commercially

available techniques such as FACS and MCAS. They go on to teach that since CD4 is expressed in developing thymocytes and T lymphocytes, the marker can be used in a variety of cells and cell lines. *Gaines et al* use a CMV promoter/enhancer, do not use a tissue specific promoter or teach that the heterologous gene could be an angiogenesis factor.

Griscelli et al teach a method of specifically targeting a therapeutic cardiac gene to ventricle cardiomyocytes comprising an adenoviral vector containing a ventricle-specific MLC-2v promoter, which could specifically targeting the gene of interest to be expressed in cardiac muscle in vitro and in vivo (abstract), and they teach that the MLC-2v is *more* specific than the MHC promoter as taught by *Klug et al* (right column, page 1920). *Griscelli et al* do not specify that the therapeutic gene is an angiogenesis factor such as VEGF.

However, before the instant effective filing date, *Mack et al* teach that a polynucleotide encoding a potent angiogenic mediator such as VEGF could be delivered to ischemic region of the myocardium along with other cardiac surgical procedure to enhance collateral vessel formation and improve regional perfusion and function, thus improve the outcome of the treatment.

Wolfgang-M et al teach that when combining the MLC-2v promoter with the CMV enhancer, the ventricular expression of the marker gene luciferase increased two-fold and 15-fold *in vitro* and *in vivo* respectively.

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the methods taught by *Klug et al, Gaines et al,*

Wolfgang-M et al, Griscelli et al, and *Mack et al* by combining the cardiac-specific promoter construct with the pIRES-CD4t construct and substituting the MLC-2v with the MLC-2v/CMV enhancer in the construct, and use an angiogenesis factor such as the therapeutic protein with a reasonable expectation of success. The ordinary skilled artisan would have been motivated to make the modification because the combined construct offers more efficient transgene expression that would be desirable for therapeutic gene delivery and using either or both antibiotic resistance gene and/or CD4 for cardiomyocyte selection. The ordinary skilled artisan would have been motivated to modify the claimed invention because the improved construct would be more efficient for cell purification and transgene expression, and cardiomyocytes expressing a therapeutic protein VEGF would provide additional means for repairing damaged myocardium with both therapeutic myocardiac cells and therapeutic angiogenic factors enhancing new vessel regeneration. Given the numerous techniques known in the art for refinement of cardiac-restricted gene expression and cell selection, these limitations would fall within the bounds of the optimization. Thus, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

The prior rejection of claims 7 and 8 under 35 U.S.C. 103(a) as being unpatentable over *Klug et al* (J Clin Invest 1996;98:216-24), *Gaines et al* (IDS, Biotechniques 1999;26:683-8), *Wolfgang-M et al* (J Mol Cell Cardiol 1997;29(5):A125), and *Griscelli et al* (Hum Gene Ther 1998;9:1919-28), as applied to Claims 1-6, 10, 12,

13, 15-17, and 32 above, and further in view of *Graham et al* (US 6,080,569) is withdrawn in view of claim amendment and a new ground of rejection that follows.

The prior rejection of Claim 14 (c) (d) under 35 U.S.C. 103(a) as being unpatentable over *Klug et al* (J Clin Invest 1996;98:216-24), *Gaines et al* (IDS, Biotechniques 1999;26:683-8), *Wolfgang-M et al* (J Mol Cell Cardiol 1997;29(5):A125), *Griscelli et al* (Hum Gene Ther 1998;9:1919-28), and *Graham et al* (US 6,080,569), as applied to Claims 1-8, 10, 12, 13, 15-17, and 32 above, and further in view of *Gainer et al* (Transplant 1998;66:194-9), and *Lallemand et al* (Transgenic Res 1998;7:105-12) is withdrawn in view of claim amendment and a new ground of rejection that follows.

Claims 34 (C-E), 35-46, and 50 are newly rejected under 35 U.S.C. 103(a) as being unpatentable over *Klug et al* (J Clin Invest 1996;98:216-24), *Gaines et al* (IDS, Biotechniques 1999;26:683-8), *Griscelli et al* (Hum Gene Ther 1998;9:1919-28), *Wolfgang-M et al* (J Mol Cell Cardiol 1997;29(5):A125), and *Mack et al* (J Thorac Cardiovasc Surg 1998;115:168-76) as applied to Claims 34 (A, B), 35, 38, 39, 40, 42, 46, and 50 above, and further in view of *Graham et al* (US 6,080,569), *Gainer et al* (Transplant 1998;66:194-9), and *Lallemand et al* (Transgenic Res 1998;7:105-12).

The combined teachings of *Klug et al*, *Gaines et al, Wolfgang-M et al, Griscelli et al,* and *Mack et al* do not teach to include an immunosuppressive protein CTLA4-Ig or a promoter constitutively operative in a mammalian ES cells such as PGK promoter, or an antibiotic selection marker gene that is flanked by a pair of LoxP sequences.

However, before the instant effective filing date, *Gainer et al* teach CTLA4 can suppress graft rejection response and prolong the survival of transplanted islet cells (CTLA4 transfected, abstract); *Gramham et al* teach that an adenoviral expression construct (figures 3 & 4) comprising a neomycin resistance gene along with the viral packaging signal flanked by two loxP sites, and upon infection of cells that express the Cre recombinase, the neo^r gene and packaging signal would be excised. They illustrated with the example how such system could be used to include or delete certain elements of the construct as needed (fig. 10). *Lallemand et al* teach a PGK promoter can be used in conjunction with the LoxP construct in genetic manipulation of stem cells, particularly to remove the 'floxed' selection cassettes such as neo gene that may influence the transcription of neighboring loci and interfere with the targeted phenotype (paragraph bridging pages 105-106). The PGK promoter activates very early in the pluripotent progenitor cells and displays ubiquitous expression (2^{nd} paragraph, page 106, and page 109), thus, suitable for genetic modification of stem cells.

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the methods taught by *Klug et al, Gaines et al, Griscelli et al, Wolfgang-M et al, Mack et al* by including a CTLA4 gene and PGK promoter as taught by *Gainer et al,* and *Graham et al*, and *Lallemand et al* in the MLC-2v-pIRES-CD4t-loxp construct for manipulation of pluripotent progenitor cells and for preventing graft rejection with a reasonable expectation of success. The ordinary skilled artisan would have been motivated to modify the claimed invention when using the pluripotent stem cells as therapeutic cells for transplantation because including a

CTLA4 in the construct would enhance the survival of cells after transplantation and because it is known in the art that the neo resistance gene is desirable for selection, and undesirable to be present in a therapeutic composition, thus including a PGK promoter in the construct would gain control over the timing of transgene expression. Thus, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Response to Arguments

Since applicants responded to the previous rejections under 35 U.S.C. 103(a) as a whole in the 1/20/04 response, thus, the Office would address the arguments accordingly.

Applicants first argue that the cited references do not describe or suggest each and every limitation of the present claims, and at least the recitation of "at least one IRES operatively linked to at least one polynucleotide encoding an angiogenesis factor" is not described or suggested by any reference.

The argument has been fully considered but found not persuasive. This because as discussed above, the recitation was taught by the combined teachings of *Klug et al, Gaines et al, Wolfgang-M et al, and Griscelli et al* with that of *Mack et al.* It appears that Applicants are arguing that the cited references do not expressly suggest the claimed invention. However, it is well established in case law that a reference must be considered not only for what it expressly teaches, but also for what it fairly suggests. In re Burkel, 201 USPQ 67 (CCPA 1979). Furthermore, in the determination of

obviousness, the state of the art as well as the level of skill of those in the art are important factors to be considered. The teaching of the cited references must be viewed in light of these factors. In the instant case, at the time of instant effective filing date, it is well known in the art that the IRES could be built-in an expression cassette for coordinating expression of multiple heterologous genes simultaneously as taught by *Gaines et al*, and therapeutic cardiac genes such as angiogenesis factor VEGF could be used together with other cardiological procedure to improve the outcome of the myocardial function in patients with heart disease as taught by *Mack et al*, and generating pure population of cardiomyocytes from genetically manipulated ES cells for cardiac transplantation is also known in the art as taught by *Klug et al*, thus, it would have fairly suggested to the reasonably skilled in the art to include an IRES-VEGF in the ES cell manipulation construct with reasonable expectation of success.

Applicants then argue that the instant invention provides a result that is not expected by the skilled artisan in the cited references. Applicants argue that the specification has shown that cells having the properties of ventricular cardiac cells are obtained whereas *Klug et al* shows expression of both atrium-specific MLC-2a and ventricle-specific MLC-2v in about equal proportion, and *Griscelli et al* provides only about 67% purification using fluorescence-activated cell sorting (FACS). Thus, a mixed population of cells would be expected by the teachings of cited art.

The arguments are fully considered but found not persuasive for reasons of record and following.

As discussed in the foregoing sections, *Klug et al* clearly teach that selection for a particular subtype of cardiac cells (ventricular or atrial) is a matter of refinement, and could be achieved by selecting for a particular promoter (page 222, 3rd paragraph), and they have shown that MLC-2v would select for ventricular cardiac cells (fig. 3, top panel). As to the purity of the selected population, *Griscelli et al* teach that three rounds of magnetic cell sorting yield > 90% CD4+ cell population (abstract). Moreover, the specification teaches the same method of cell sorting, and the claims do not require that only ventricular cells be selected nor the purity of the selected population. Claim 50 only requires transforming pluripotent precursor cells with the expression cassette. Accordingly, the combined teachings of the cited art meet each and every limitation of the claims, and the claimed invention is obvious over combined teachings of cited prior art.

Conclusion

No claim is allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not

mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to **Q. Janice Li** whose telephone number is 571-272-0730. The examiner can normally be reached on 9:30 am - 7 p.m., Monday through Friday, except every other Wednesday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, **Amy Nelson** can be reached on 571-272-0804. The fax numbers for the organization where this application or proceeding is assigned are **703-872-9306**.

Any inquiry of formal matters can be directed to the patent analyst, **Dianiece Jacobs**, whose telephone number is (571) 272-0532.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist **Rena Jones** whose telephone number is **571-272-0571**.

JANICE LI
PATENT EXAMINER

Q. Janice Li
Patent Examiner
Art Unit 1632

QJL
April 1, 2004